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INSECT DEVELOPMENT ANALYZED BY EXPERIMENTAL METHODS: A REVIEW

PART I. EMBRYONIC STAGES[1]

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[1] This paper was prepared in the biological laboratories of the University of Rochester and of Cornell University.

I. INTRODUCTION

This is the first of two papers having as their joint purpose an analytical review of the available experimental data on all the phases of insect ontogeny. Development from egg to adult is physiologically a continuous process regardless of the form changes involved. Nevertheless we find it convenient to treat the subject under two arbitrary subheads. The present paper is concerned primarily with development within the egg, while the second will deal with development after eclosion from the egg.[2] Recent reviews in English make it unnecessary to cover in detail such general topics as sex determination, determination of types of individuals, action of genes, and chemical changes during development. However, some of the more recent papers are mentioned when they aid in maintaining a proper perspective.

Although experimental insect embryology is still in a very early stage in comparison with experimental vertebrate embryology, it has reached a point where the literature is sufficiently extensive and the results sufficiently definite to make a résumé in English seem desirable. Such a résumé seems especially valuable because our view-point has changed considerably since Morgan (1927) and Schleip (1929) reviewed certain of the outstanding papers.

Most of the important data on the embryonic stages are of recent date. Historically the first experiments were those of Wheeler (1889) and Megušar (1906) on gravitational effect by the inversion of eggs. The first important experiments were those of Hegner on beetle eggs. In 1908 he studied the effects of puncturing the egg and allowing part of the contents to flow out; in 1909 the effects of centrifugal force, and in 1911 the effects of killing regions of the egg with a hot needle. A lapse of about fifteen years followed, during which the little experimental data came from genetical and cytological investigations. Then, with the work of Reith (1925), Seidel (1926) and Pauli (1927), began the series of experiments that will fill the bulk of this paper. To Seidel and his students we owe most of our knowledge of the vital processes occurring in the insect egg.

[2] Berlese's theory which attempts to harmonize the great diversity found in the ontogeny of the various groups of insects will be discussed in Part II.

For our purposes we shall regard *'determination'* as the process of primary chemo-differentiation that 'sets the course' along which a given region of the egg is to develop, *i.e.,* determines its fate. Determination lays the invisible foundation for morphological differentiation. It may, and probably always does, vary in degree, becoming increasingly more strict as development proceeds, and so may also be expressed as 'a limitation of potencies.' *Differentiation* has been aptly defined by Schnetter (1934a) as "visible distinctive configuration." It is the realization, as visible development, of the capacities of a region acquired by earlier or simultaneous chemo-differentiation.

The degree of determination existing in the egg at a given stage of development is reflected in the ability of the egg to readjust itself toward the production of a normal embryo after a defect is imposed upon it. The more fixed the determination, the less the power of regulation. Insect eggs may accordingly be grouped into (1) *indeterminate,* (2) *incompletely determinate* and (3) *determinate* types. An indeterminate type of egg is one in which the parts of the embryo are not predetermined at the time of fertilization, and hence an egg with great regulative powers under experimental conditions. A determinate type of egg is one in which the parts are wholly predetermined before or during fertilization, and hence an egg with little or no regulative power and given to mosaic formation under experimental conditions. An incompletely determinate type of egg is intermediate between these two. A graded series is found from the highly indeterminate eggs in certain of the lower insect orders through incompletely determinate eggs to the completely determinate eggs of the higher Diptera. This indeterminate-determinate series will be discussed in detail later.

Space will not permit a detailed discussion of the morphological course of insect embryogeny. Good accounts are given in recent entomological text-books, *e.g.,* Imms (1934), Weber (1933) and Snodgrass (1935). Such descriptive notes as seem imperative will be given under the forms as discussed.

II. EXPERIMENTAL MATERIALS AND METHODS

The forms experimented upon include Orthoptera, Odonata, Coleoptera, Diptera and Hymenoptera. Both aquatic and ter-

restrial types have been used; study of the development of the latter is frequently facilitated by immersion in water. Observations on living eggs are possible when they are naturally transparent or translucent and in some cases after removal of the chorion (egg-shell) (*e.g.,* Slifer 1932a, Child & Howland 1933). In experimental studies normal eggs are always used as controls and in some problems 'experimental controls' are also used (*e.g.,* partially *versus* completely constricted eggs). The experimental techniques that have been used may be summarized as follows. [References in italics give methods in detail.]

1. **Cauterization**: (a) Killing a small region or several regions with a hot needle either to produce a minute scar or to eliminate certain areas [Hegner 1911 (Chrysomelid beetles); Reith 1925 (*Musca,* the House Fly), 1931b (*Camponotus,* an ant) and 1935 (*Sitona,* a weevil); *Seidel* 1926, *1929b* (*Platycnemis,* a damsel fly); Strasburger 1934 (*Calliphora,* a blow fly); *Howland & Robertson 1934* (*Drosophila*), Oka 1934 (*Gryllus,* a cricket)]. (b) Light cauterization to produce localized contractions of the yolk system [Seidel 1934 (*Platycnemis*)]. (c) Unilateral heating with a microcauterizer to produce minute splits by unequal expansion of the egg materials [Seidel 1928, 1929b (*Platycnemis*)]. (All workers except Hegner used electrically-heated needles, some with micro-manipulator control.)

2. **Irradiation with ultra-violet light** to (a) kill selected nuclei, (b) kill certain areas of the egg, (c) cause temporary changes in the cytoplasm, (d) produce a minute scar, or (e) observe the effect of *in toto* irradiation of oriented eggs [*a-d Seidel* 1929 ff., *1932* (*Platycnemis*); *e Geigy* 1926 ff., *1931b* (*Drosophila*)].

3. **Puncturing** the egg with a cold needle, either to allow part of the egg contents to flow out, or to injure, divide or alter the germ band [Hegner 1908 (Chrysomelid beetles); *Krause 1934* (*Tachycines,* a camel cricket); Sonnenblick 1934 (*Drosophila*); Howland & Child 1935 (*Drosophila*)].

4. **Producing yolk-fissures** by bending the egg [Seidel 1929a (*Platycnemis*)].

5. **Constricting** the egg (completely or incompletely) at various points with a fine hair [Seidel 1926 ff. (*Platycnemis*);

Pauli 1927 (*Calliphora* and *Musca*); Rostand 1927 (*Calliphora*); Reith 1931b (*Camponotus*); *Schnetter 1934b* (*Apis,* the Honey Bee); Brauer & Taylor 1934 (Bruchid beetle)].

6. **Centrifuging** the egg in various positions [*Hegner 1909* (Chrysomelid beetles); Clément 1917, 1921 (*Bombyx,* the Silk Moth); *Pauli 1927* (*Calliphora* and *Musca*); Reith 1932b (*Camponotus*)]. One might also include here the inversion experiments of Wheeler (1889), Megušar (1906) and Hegner (1909).

7. **Modification of environmental factors** [Slifer 1934 (anisotonic salt solutions on *Melanoplus,* a grasshopper); Bodine *et al.* (temperature, oxygen tensions, cyanide, etc., on *Melanoplus*), and various workers with temperature, humidity, nutrition, general irradiation with various rays (alpha rays (*Hanson & Heys 1933*), mitogenetic rays (*Wolff & Ras 1934*), x-rays (Henshaw 1934, Smith 1935)), etc., on various insects].

8. **Genetic analysis** of 'normal' mosaics, intersexes and gynandromorphs in studies on fertilization, cell-lineage, sex determination and to a lesser extent organ formation [Goldschmidt 1917 ff. (moths, *Lymantria* and *Bombyx*); Sturtevant 1929 and Dobzhansky 1931 (*Drosophila*); Whiting *et al.* 1924 ff. (*Habrobracon,* a wasp)].

In regard to techniques that have been used upon other invertebrates and upon vertebrates it is of interest to note that (1) separation of early blastomeres is of course impossible due to the superficial cleavage, but this aspect is covered by the above methods nos. *2, 5* and *8*; (2) vital staining for marking egg-regions has been tried unsuccessfully by Seidel (he did not remove the chorion) and no. *2d* above had to be used instead; and (3) tissue culture methods, parabiotic twinning and operative technique in the sense of transplantations have not been successfully accomplished with insect eggs. The tough chorion together with the turgidity and fluidity of the egg contents hinder manipulation.

III. ORIGIN OF POLARITY AND SYMMETRY

Insect eggs vary greatly in form but may be broadly classified as either radially or bilaterally symmetrical. In either case the eggs may be laid in definite or random positions depending on the species. In bilaterally symmetrical eggs the axes of the presumptive embryo may be externally evident.

1. **Hallez's Law of Orientation:** This 'law' is based on the observation that the mature egg within the ovary lies in such a position that all three axes of the presumptive embryo are oriented coincidently with those of the mother. From this it follows that the embryonic axes are determined in the egg before laying. Although this is certainly a general rule it is not yet proven to be universal. In the radially symmetrical egg of the bug *Pyrrhochoris* Seidel (1924) reports that the longitudinal embryonic axis may vary from being coincident with the longitudinal axis of the egg to being transverse to it. Also there are certain insects (*e.g.,* Melanoplus, Slifer 1932a) in which it is reported that the embryo begins to develop in the reverse position with the head towards the micropylar opening at the posterior end of the egg but that the 'normal' position is attained by a later reversal of the embryo during blastokinesis.

Seidel (1929a) reports that frontal doubling, induced by the production of splits in the egg at the beginning of cleavage and resulting in the formation of mirror-image symmetrical twins within the egg, indicates the determination of a dorso-ventral axis (as well as other axes) at this early stage in the development of *Platycnemis.* Mirror images result from the inversion of one of the three axes in the formation of one partner; this presupposes the existence of polarity at least in the inverting plane. Frontal and lateral doubling have also been induced by splits in the germ band of *Tachycines* (Krause 1934).

2. **The position of the micropyle** is determined by the formation of the egg-shell during growth of the oöcyte. It is usually situated at the center of the anterior end of the egg but may be on one side (Weber, 1933, p. 488) or even at the posterior end (Slifer 1932a). If the point of entrance or course of the sperm is concerned in the determination of the embryonic axes then these in turn are more or less fixed by the position of the micropyle and hence influenced by ovarian factors.

3. **Visible bilaterality** is frequently evident in the external structure and form of the mature egg. A corresponding visible internal bilaterality is sometimes shown by the constant position of the "richtungsplasma" (place where the maturation divisions occur) on the dorsal or ventral mid-line of the egg (Schnetter

1934a, Strasburger 1934). Hirschler (1933) has demonstrated by intra-vitam staining that the original radial symmetry of the egg becomes obviously bilateral during deposition of the yolk in the beetle *Cicindela* but he did not study the relation of this symmetry to that of the future germ band; the two may be coincident.

4. **Germ-Tract Determinant** (germ-plasm, germ-line, pole-cells, pole-disc, Polscheibe, Keimbahn): There is frequently an area of distinct appearance at the posterior end of the unfertilized egg. The cleavage nuclei which enter this region are destined to give rise to the definitive germ cells of the embryo. This area frequently contains numerous granules that stain conspicuously. Huettner (1923) shows that they are neither small yolk granules nor mitochondria; presumably they are merely byproducts of the 'germ-plasm.' In any event the area is most probably determined by extra-oval factors and does not act in the determination of the embryonic axes.

5. **Gravity and centrifugal force:** Wheeler (1889) and Hegner (1909) report that the position of the eggs of the Cockroach and Chrysomelid beetles has no effect on development. The single reported exception is the beetle *Hydrophilus* in which Megušar (1906) reports that if the aquatic egg-capsules are inverted development is retarded and the few larvæ which hatch are deformed and soon die (the embryos, however, were in their normal position within the eggs). His results, based on only two capsules of eggs, are inconclusive since he did not consider the modification produced in factors other than gravity, for instance the inversion of air-water relations.

Hegner (1909), Pauli (1927) and Reith (1932b) report that the orientation of embryos produced by centrifuged eggs is unaffected although the visible materials are redistributed and stratified. Centrifugal force has much less effect on eggs within the ovaries (Hegner 1909, Clément 1917, 1921), either due to less effect on growing or mature oöcytes or to a restitution within the oöcyte.

From the above fragmentary notes it is evident that the fundamental processes underlying the determination of polarity and symmetry are still a matter of conjecture. In bilaterally sym-

metrical eggs, at least, both polarity and symmetry must be determined during growth of the oöcyte. The development of polyembryonic eggs (certain parasitic Hymenoptera) might throw some light on this question but the available data can not be analyzed from this view-point. Cappe de Baillon's (1925b) data clearly suggest to us that this early determination of the fundamental axes begins at or near the anterior end and progresses posteriorly (see Section VI).

IV. FERTILIZATION

1. **Activity of the spermatazoa:** Activated sperm have an undulatory movement but it is not known whether this movement functions during penetration of the insect egg or only furnishes motile power to pass through the micropyle. The tail, in all reports we have noted for insects, is carried into the egg. How the sperm move through the interior of the egg after entrance is unknown, but it is of interest to note that Huettner (1927) observes their distribution throughout the egg in abnormally highly polyspermatic eggs of *Drosophila*. Also little is known concerning the reactions of the sperm themselves, but Howland (1932) reports that in *Drosophila* the sperm are highly sensitive to the constitution of the surrounding medium and easily killed.

2. **Monospermy and polyspermy:** In some insects only a single sperm can be found within a fertilized egg (Johannsen 1929) but in most insects several or many sperm enter. Huettner (1924, 1927) reports that supernumerary sperm in *Drosophila* eggs degenerate, only rarely forming mitotic figures. If, however, the degree of polyspermy is too great it may lead to disturbances which prevent further development. These disturbances are usually due to disorganization of the maturation divisions by sperm which enter this region of the egg. Such sperm may form uni-, bi- or multipolar spindles, or even enter normal spindles to form multipolar figures. This condition may or may not become adjusted. In binucleate moth eggs Doncaster (1914) and Goldschmidt & Katsuki (1928) report that each nucleus fuses with a sperm. Genetic evidence shows that the same is true for some binucleate eggs of the wasp *Habrobracon* (Whiting 1934). In no case is there any evidence of a sperm

functioning except after uniting with an egg nucleus, but Huettner (1927) postulates that such is possible and shows that it might conceivably be followed by normal development.

3. **Selective fertilization:** In the true sense this is unknown in insects although it may be simulated in some instances by a partial or complete mortality of one of the expected types. This is presumably the case in sex determination in the Hymenoptera where according to Whiting's theory (1933) biparental progenies should be composed of diploid males and females in equal number. He shows by genetic tests with sex-linked genes and by an inverse correlation of biparental males with total progeny and with egg-hatchability, that the fusion of like gametes is usually lethal—fusion of unlike gametes giving females (normal males are haploid and result from parthenogenesis). A presumed case of selective fertilization in *Drosophila* has been shown due to the necessity of the dominant allelomorph at some time during development even though it be expelled into a polar body (Morgan 1927, p. 58).

4. **Activation of the egg by the sperm:** Ripe insect eggs are usually found to be in the metaphase of the first maturation division and to depend on the entrance of the sperm or the induction of parthenogenesis to continue further (Huettner 1924, Johannsen 1929, and others). In normally parthenogenetic species there is no such cessation at this point, and even in the grasshopper *Melanoplus* which is normally fertilized at this stage King & Slifer (1934) report that there is no visible delay in unfertilized eggs and that the maturation divisions proceed to completion at very nearly the same rate as in fertilized eggs. Accordingly the sperm does not necessarily have an activating function even in eggs which ordinarily undergo fertilization.

5. **Relation of fertilization to cleavage and later development:** It was pointed out above that since the point of entrance and path of the sperm are probably constant in any one species due to the position of the micropyle, it is uncertain whether they bear any relation to the origin of the orientation of the embryo. The only cases in which any part of fertilization is thought to have a bearing on development, other than initiating completion of the maturation divisions, is in incompletely determinate eggs. In this type of egg Reith (1931b, 1935) reports that induction of

the visible zonation of the cortical layer of the ant egg and a similar but not visible determination in the beetle egg start immediately after the beginning of cleavage. How this activation of the activation center is brought about and whether it is caused by the entrance of the sperm, the fusion of the pronuclei or some other factor are unknown.

6. **Fertilization membranes** are seldom mentioned for insects. In the Silk Worm Bataillon & Su (1931a, 1933) report that a strong fertilization membrane is detached from the egg following induced parthenogenesis and in the first brood of two-brooded stocks but not in the second brood or in single-brooded stocks. The significance of this variation is not known.

7. **Centrioles** are present in the eggs of some species, absent from others. Huettner (1933) shows that they are absent from the maturation figures of the egg of *Drosophila* but that they appear at the first cleavage (from the sperm?) and thereafter have a continuous history throughout development. Nachtsheim (1913) believes that in the Honey Bee they are derived from the egg since supernumerary sperm form anastral spindles whereas cleavage cells have conspicuous centrioles. Bataillon & Su (1931b, 1933) report that in the Silk Worm egg they do not appear except from the sperm, and that they are never found in parthenogenetic eggs. Their absence from these parthenogenetic eggs is frequently accompanied by highly abnormal mitotic figures.

8. **Binucleate eggs:** These may and presumably do arise from two different causes: (1) the functioning of two maturation nuclei of a single egg, and (2) the fusion of two eggs. Strangely enough the fusion of two eggs does not result in a giant egg which in turn produces a giant individual. Morgan (1927, p. 466) suggests that two oöcytes may fuse early in growth, and then having only the nourishment of a single egg not grow beyond normal size.

On purely genetic evidence Whiting (1924, 1934) postulates the functioning of two maturation nuclei of the egg (pronuclei) to account for haploid mosaic males in the wasp *Habrobracon*. On similar data he shows that in the case of fertilized eggs either one or both of such nuclei may fuse with a sperm. Detailed

analysis of his data leads us to accept his postulate that the two nuclei are derived from the maturation nuclei of a single egg rather than from fusion of two oöcytes. Goldschmidt & Katsuki (1928) report both the cytology and genetics of a similar case in the Silk Worm Moth. Here it is produced by a recessive gene tending to form binucleate eggs, and the genetic evidence proves conclusively that two functional nuclei come from the egg in all cases. Doncaster (1914) had already found binucleate moth eggs cytologically, each nucleus accompanied by three polar bodies and being fertilized by a separate sperm nucleus. This is conclusive proof that in this case we are dealing with an egg containing two diploid nuclei, not with an egg in which two reduced (maturation) nuclei are functioning. Also Morgan and his coworkers (1914, 1919, 1923) show genetically that binucleate eggs are the most probable explanation of certain exceptional specimens of *Drosophila.*

In addition to the above cases there are the following definitely referred by the authors to the fusion of two oöcytes. In the wasp *Copidosoma* Hegner (1914b) claims that two oöcytes regularly fuse, one furnishing the nucleus of the mature egg, the other furnishing the germ-tract determinant. Patterson (1917, 1921, 1927) in dealing with closely related species does not mention this claim nor cite this paper of Hegner's. In agreement with earlier authors he describes the mature egg as arising from a single oöcyte, the germ-tract determinant originating from the nucleolus. Cappe de Baillon (1925, 1927) is the strong proponent of oöcyte fusion to give bi- and tri-nucleate eggs. According to him the determination process in the cortical layer of the egg of the phasmid *Carausius morosus* begins during growth of the oöcyte. If the fusion occurs sufficiently early or if the fusion planes happen to coincide a normal embryo will result, but if the fusion planes do not coincide an egg with two separately determined plasma regions will result. He finds all stages from normal embryos to two separate embryos within one egg. To corroborate this hypothesis he, as well as Zakolska (1917) and Tur (1920), has found various stages in the fusion of growing oöcytes within the ovary. Von Lengerken (1928) accepts Cappe de Baillon's hypothesis and describes several presumably additional

cases from the beetles *Carabus* and *Lucanus*. In these non-parthenogenic forms oöcyte fusion may result in the production of gynandromorphs.

Whether or not the origin is the same in all cases there can be no doubt that binucleate eggs occur in such widely separated groups as phasmids, moths, beetles, wasps and flies. In all the above cases the egg is reported as responsible, supernumerary sperm never functioning except when they unite with an egg nucleus. [Huettner (1927) shows that in *Drosophila* supernumerary sperm might conceivably function without fusion but his point is yet unproven.]

9. **Trinucleate eggs**: As with binucleate eggs there are at least two possible methods of origin of trinucleate eggs. Cappe de Baillon (1927) figures trinucleate eggs of *Carausius,* his figures indicating that they have arisen by the fusion of three separate oöcytes. Whiting (1934) postulates trinucleate eggs in *Habrobracon* because mosaic males bred from virgin females sometimes show three or even four possible combinations of maternal characters. He also reports one gynandromorph thought to have arisen from a trinucleate egg one nucleus of which became fertilized.

10. **Parthenogenesis**: There are some insects in which all individuals develop parthenogenetically from non-reduced eggs (giving only females); such parthenogenesis may be either constant or cyclic but is always obligatory. In Hymenoptera and certain other forms reduced but unfertilized eggs normally produce males. In other insects parthenogenetic development of reduced eggs occurs occasionally or can be induced. A detailed treatment is given by Weber (1933, p. 514) so only a representative set of cases is cited here.

Goldschmidt (1917) gives a detailed analysis of a case of facultative parthenogenesis in the Gypsy Moth. Clément (1917, 1921) reports that it is sometimes induced by centrifuging Silk Worm eggs. Bataillon & Su (1931a) report that it can be induced by chloroform or weak acids in Silk Worm eggs and that it frequently results in an activation superior to that of fertilization. Harrison (1933) and others have reported that it is sometimes induced by attempted hybridization. A peculiar case is reported

by Shull (1930a) for aphids. Here the mode of reproduction of an individual is determined before birth by external factors (light & temperature). It is the determination of the mode of reproduction of an individual by the action of factors upon its mother rather than upon the generation affected.

Slifer & King (1932) and King & Slifer (1934) report that in unfertilized eggs of the grasshopper *Melanoplus* the maturation divisions are completed at nearly the same rate as in fertilized eggs. The embryos developing from these eggs frequently contain both haploid and diploid cells. This, as well as genetic evidence from parthenogenesis in the grouse locusts (King & Slifer 1933), indicates that the maturation divisions are normal and that there is no fusion of a polar body nucleus with the true egg nucleus but that during cleavage there is a doubling of chromosomes without separation into two nuclei. The animals reared to maturity were all females. This, coupled with the fact that from thousands of unfertilized eggs several hundreds hatched but only about twenty lived to maturity, seemingly indicates that only those individuals which successfully attain a diploid condition live to maturity. Since the second generation gave results similar to the first the high mortality must be ascribed to the haploid condition itself, not to uncovered lethal genes.

V. CLEAVAGE AND BLASTODERM FORMATION

The insect egg is made up of a central yolk mass enmeshed in a slight cytoplasmic reticulum and surrounded by a relatively thin layer of dense cytoplasm—the cortical layer or periplasm. At the time of union of the sperm and egg nucleus the latter lies somewhere within the yolk region of the egg (its position probably being constant in any particular species). Due to this position and the centrolecithal yolk distribution, cleavage is of the superficial type in all the species treated in this review, *i.e.,* cleavage consists of nuclear divisions accompanied by division of only the small cytoplasmic area immediately around the nucleus (the so-called 'protoplasmic island'), true cell formation not occurring until the cleavage nuclei penetrate the cortical layer where they give rise to the multicellular blastoderm. [This type of cleavage is peculiar to the arthropods but is not universal among the insects.]

No development occurs if the early cleavage nuclei are killed by cauterization (Reith 1925, 1931b, Pauli 1927)—the formation of an enucleate 'blastoderm' in unfertilized eggs of *Phragmatobia* hybrids is an apparent exception (Seiler 1924, see Section V–B). Nuclei must enter the region of the activation center (*q. v.*) before differentiation of the germ band can occur in *Platycnemis* (Seidel 1932), but in *Camponotus* this center functions before it becomes nucleated (Reith 1932b). The cleavage divisions are not inseparably bound up with the early developmental processes since they continue for some time after elimination of the activation center (Seidel 1929b, Reith 1931b) or after blocking of all development by x-irradiation (Henshaw 1934).

A. Specialization of Nuclei

The cleavage nuclei are usually but not always indeterminate or totipotent at least until blastoderm formation (Sturtevant 1929, Seidel 1932). In *Platycnemis* Seidel (1932) reports that neither abnormal or delayed nuclear distribution nor elimination of one of the first two nuclei prevents formation of a normal embryo, nor does a decreased number of nuclei necessarily delay the onset of determination and differentiation. Equipotentiality of the nuclei is also indicated in the experimental results of Hegner (1908), Reith (1925) and Pauli (1927).

At first the cleavage divisions occur synchronously,[3] but sooner or later this synchrony ceases and heterochronous divisions begin, usually during blastoderm formation. Heterochronism may be regarded as the first indication of nuclear differentiation, but in some cases it is preceded by vitellophags being left behind in the yolk.[4] In *Platycnemis* the nuclei reach the surface between the fifth and sixth divisions but beterochronism does not set in until the tenth division. Seidel (1929b) reports that there is some indication that cleavage ceases after the last synchronous division when the activation center (*q. v.*) is eliminated. In *Ephestia*

[3] Synchrony of cleavage divisions has been reported in Apterygota, Orthoptera, Dermaptera, Aphidæ, Odonata, Coleoptera, Lepidoptera, Hymenoptera and Diptera (see Schnetter, 1934a, table 12). If the onset of a different division rhythm for the germ-tract nuclei be counted the number of synchronous divisions varies from three in *Miastor* to ten in *Apis*.

[4] Ordinarily the vitellophags arise from nuclei that migrate back into the yolk from the blastoderm. For a tabular summary of the data on vitellophag origin see Sehl, 1931, p. 570.

and *Apis* heterochronism begins with the tenth and eleventh divisions respectively, when the nuclei enter the cortical cytoplasm. Schnetter (1934a) describes the heterochronous division in *Apis* as proceeding in 'waves,' the mitoses occurring first in a definite region of the egg (see Section VII–B) and spreading successively to neighboring nuclei. In the Diptera those cleavage nuclei which happen to enter the germ-tract determinant region at the posterior pole of the egg cease dividing synchronously with the other nuclei as soon as they become segregated as 'pole cells,' although synchrony continues independently within each set of nuclei at a different rate from that in the other set. In the fly *Sciara* DuBois (1932) reports the elimination of whole chromosomes from all nuclei at the fifth division and prior to the segregation of the germ-tract nuclei, and further elimination during the seventh to ninth divisions from the remaining (somatic) nuclei after germ-cell segregation. In *Miastor* Huettner (1934) reports the elimination of three-fourths of the total number of chromosomes from the somatic nuclei during the third and fourth cleavage divisions and after the segregation of the germ cells (reduction from octoploid to diploid condition). In neither case is the division synchrony affected by this elimination. In *Camponotus* Reith (1931b) reports that after early destruction of the activation center, the usual zonation of the cortical layer does not occur and no true blastoderm is formed, yet the cleavage nuclei differentiate into visibly distinct types (embryonic and extra-embryonic). At present these various types of nuclear differentiation cannot be attributed to a common factor. Cytoplasmic influence seems operative in germ-cell differentiation in the Diptera and possibly in the described behavior in *Camponotus;* the developmental centers (Section VII) through their effect on cytoplasm, other oöplasmic constituents or nuclear migration, may influence division heterochronism in *Platycnemis* and *Apis;* whereas intrinsic factors in the nuclei themselves may be more directly responsible for chromosome elimination in the lower Diptera and for vitellophag formation.

B. Migration of Nuclei

In *Platycnemis* Seidel (1932) reports that the first four cleavage nuclei are oriented at intervals along the longitudinal axis (Fig.

1a). He says this is due to a strong repulsion tendency of the mitotic figures against one another and against the surface of the egg, the tendency becoming reduced as the nuclei and protoplasmic islands become smaller. He observed occasional cases of atypical distribution which he thinks due to an absence or failure of this tendency, but one would expect visible abnormalities in mitoses if such were true. Seidel also reports that the activation center (Fig. 1 a–b) is not concerned in nuclear migration since the distribution of the nuclei occurs almost or quite normally when this center is completely constricted off from the rest of the egg.

In the butterfly *Pieris* Eastham (1927) shows that the cytoplasm of the protoplasmic islands extends into the cytoplasmic reticulum of the yolk in the form of long tapering strands distributed around the nucleus. Via these strands the cytoplasm of the protoplasmic islands is continuous with that of other islands and of the cortical layer. Therefore the egg is a true syncytium with each nucleus lying in the center of a small cytoplasmic area. As cleavage begins the mitotic figures are oriented at random and result in a cluster of nuclei. The first indication of their migration is the formation of a small hollow sphere of nuclei. In this sphere subsequent nuclear divisions occur parallel to the surface with the result that the spherical arrangement is maintained. Concerning the migration itself Eastham believes the nuclei are moved passively through the yolk by centrifugal streaming because, (1) flecks of cytoplasm are frequently seen elongated in the direction of nuclear movement; (2) the comet-like tails of the protoplasmic islands stretching out behind the migrating nuclei are also present during mitosis when the nucleus is not thought of as active; (3) as the nuclei approach the cortical layer the intervening area becomes visibly richer in cytoplasm, and (4) conversely the area within the nuclear sphere becomes poorer in cytoplasm. In addition he notes that the nuclei are first nearer the cortical layer in the lateral and anterior regions but that then the posterior nuclei move more rapidly thereby changing the shape of the nuclear sphere (first reach surface antero-ventrally). During this time each nucleus moves to the peripheral end of its protoplasmic island, and, as it enters the cortical layer, becomes

elliptical with its longitudinal axis parallel to the egg surface (evidence of pressure between nucleus and cortical layer). The comet-like tails are then drawn in and spread out to fuse with one another enclosing a thin layer of yolk peripheral to their points of fusion. This visibly distinct cytoplasmic layer is called the 'inner cortical layer.' Sehl (1931) reports fundamentally the same for the moth *Ephestia*.

In the Honey Bee Schnetter (1934a) divides the kinematic appearances into three phases: (1) first four divisions near the anterior pole with successive spindles at right angles to one another (Sach's Law) and giving first a quadrant then a hollow sphere of sixteen nuclei; (2) fifth to seventh divisions in a longitudinal direction, tangential to the surface of the elongating sphere, the center becoming almost devoid of cytoplasm, the periphery rich; and (3) migration to the cortical layer during the seventh to tenth divisions, the movement being most rapid in the widest region of the egg and toward the ventral side. The first vitellophags arise by radial division of one or two nuclei during the seventh division, and are described as moving inward against the general outward movement. He refuses to commit himself as to how the nuclei move but it certainly is not merely a repulsion tendency of the mitotic figures.

In the ant *Camponotus* and the beetle *Sitona* Reith (1931b, 1935) reports that early elimination of the activation center does not affect nuclear movements despite the fact that this center normally functions during cleavage.

In *Calliphora* Strasburger (1934) reports that an oval sphere of nuclei is formed at the sixteen-nuclei stage. During metaphase each mitotic figure lies tangential to the surface of this sphere, but during telophase, when no spindle fibers are apparent, the daughter nuclei move in a radial direction in such manner that the mitotic axis bends and the nuclei lie at an acute angle to each other until completely separated. In this connection it is seen that the position of the nuclei at the peripheral end of the protoplasmic islands is correlated with mitosis—the spindle is at the center during metaphase, at the peripheral margin during telophase. The nuclei enter the cortical layer synchronously, but this synchrony of movement is not especially important since

disturbances in it merely result in part of the embryo anlage developing sooner than the rest, a normal embryo being eventually formed. Strasburger suggests that the protoplasmic streaming is due to active movement of the protoplasmic island or its parts since in lightly cauterized eggs no such streaming is visible in non-nucleated but "uninjured" parts of the egg. However formation of the inner cortical layer is clearly a plasma streaming without nuclear influence for when the nuclei are delayed the cortical layer thickens prior to their entrance (greater ventrally than dorsally). In *Drosophila* Huettner (1935) says that there are three types of movements: (1) first eight divisions resulting in the even distribution of 256 nuclei throughout the egg; (2) movement into the cortical layer; and (3) movement of the posterior nuclei in the formation of the germ cells. He thinks that the first type of movement is "partially conditioned by the mitotic spindle," though other factors must be involved since the nuclei continue to move slightly when spindles are not present [viscosity changes concurrent with mitosis might be a factor here]. He offers no explanation of the second type, but refutes the possibility of protoplasmic streaming because of vitellophags being left behind. He thinks the third type probably due to a combination of spindle action and protoplasmic flow.

Analysis of the motivating factor or factors in these nuclear movements is difficult because of the inability to separate cause from effect in observational data. There is certainly no amœboid movement exhibited by either nucleus or protoplasmic island. The movement is not impeded by repeated mitoses during migration, in fact, Huettner (1935) says movement may in certain cases be accelerated during mitosis in *Drosophila.* The movement of the centrioles during mitosis shows that they cannot be the causative factors even though they constantly lie towards the egg-periphery in undividing nuclei (Huettner 1933, Strasburger 1934). The repulsion tendency cannot possibly function to cause centrifugal movement of the nuclear sphere.

There remain three possibilities: (1) active nuclear movement which pushes the cytoplasm in front of it and draws that behind; (2) plasma streaming which carries the nuclei along passively, and (3) some unknown attractive influence from the surface

region. The only observation that can be considered in favor of the first hypothesis is Strasburger's report that plasma streaming does not occur in non-nucleated but seemingly uninjured parts of the egg of *Calliphora*. But this is by no means conclusive evidence even for this species since he does not consider such questions as the possible failure of local activation, altered viscosity or other possible invisible physico-chemical changes in these cauterized eggs. He attempts to circumvent the difficulty of ascribing active nuclear movements to the nucleus by suggesting that the movement is due to the protoplasmic island in whole or part, but this is unsupported and also conflicts with his own data on the formation of the inner cortical layer and with Seiler's data to be discussed below. Another possibility that has not been suggested by any previous author is that the qualitative differences between the various nuclei, as expressed by the non-migration of vitellophags, might conceivably result in surface-tension phenomena around each nucleus. Such differences, arising from differences in the nuclei themselves, might possibly inaugurate the general plasma streaming and also cause the differences between the movements of various nuclear-types, perhaps largely by causing the vitellophags not to participate in the general outward movement or actually to move against it. In favor of the second hypothesis, plasma streaming, there are two positive observations. (1) Strasburger's report that when some of the nuclei anterior to the point of cauterization are delayed from entering the cortical layer in eggs of *Calliphora* the inner cortical layer is formed in those regions before the nuclei reach it. (2) Seiler's report (1924) that the eggs of *Phragmatobia* (moth) hybrids occasionally undergo 'cleavage' of the cortical layer to form a pseudo-blastoderm even though no cleavage nuclei are present in the egg. His illustrations show clearly that those areas which undergo this 'cleavage' process have a considerably thickened cortical layer. Both of these cases certainly represent a plasma streaming without nuclei, from which it is reasonable to assume that the plasma streaming is the primary factor in these movements. But plasma streaming alone leaves unexplained those cases where vitellophags are left behind in the yolk (unless the plasma streaming is local and caused by

the nuclei—its general appearance being due to a summation of local effects around each nucleus. See above). The third hypothesis is indefinite. It is included here partly as a possible answer to the occasional non-migration of vitellophags, partly as a possible source of origin of the plasma streaming (see below).

The factor or factors causing the centrifugal migration of the nuclei must be the same that bring an end to the random orientation of the mitotic figures and arrange the nuclei in the form of a hollow sphere. It would seem that only a centrifugal plasma streaming could accomplish this. But from what could such a plasma streaming originate? Certainly not directly from randomly oriented nuclei. Perhaps it is unwise to attempt delimitation to any single factor or part, but it seems possible that the stimulus might arise from the distant influence of the cortical layer. The original shape, later movements and change of shape of the nuclear sphere are not too well correlated with the original thicknesses of the various regions of the cortical layer so it can scarcely be a truly quantitative relationship. Nevertheless Schnetter (1934a, p. 161) points out that there are certain quantitative correlations in the Honey Bee egg. He describes the outward movement of the nuclei as occurring in waves passing anteriorly and posteriorly from the region of the differentiation center (*q. v.*), the nuclei first reaching the surface anteriorly and ventrally near the locus of polar-body formation, and suggests that a motivating impulse is transmitted from the differentiation center following the release of the qualitatively distinct "Richtungsplasma" into the periplasm. There are, then, two possible origins of this centrifugal movement, both hypothetical: (1) a more or less general effect from the cortical layer, or (2) qualitative differences not necessarily included in the developmental centers although sometimes correlated with the differentiation center. Either is substantiated but not proven by Strasburger's observation that light cauterization of the egg of *Calliphora* frequently results in a displacement anteriorly of the nuclear sphere. If either of these suggestions is true, then the origin of the plasma streaming is one of the first indications of the activation of the *general* dynamic egg system[5] (see Section

[5] In a sense vitellophags left behind in the yolk form an exception here as was noted in the last paragraph. But, just as they are sometimes visibly

VII–C). Whether such influence acts by changing concentration gradients or by some other means is totally unknown. In this connection we might add that Hegner (1909) has shown that the contents of Chrysomelid beetle eggs are more easily stratified by centrifugal force during late cleavage than at any time before or after. Presumably the egg contents are less viscous at this time. Also Pauli (1927) notes that after reorganization of the disarranged egg contents the nuclei come to lie near the boundary between zones or distribute themselves evenly over the surface of the egg.

It should also be noted that in the indeterminate-type eggs of *Platycnemis* and *Tachycines* further migration occurs after the nuclei are uniformly distributed over the surface. The cells assemble in two groups (ventral in *Platycnemis,* dorsal in *Tachycines*) which finally come together on the ventral side of the egg to form the embryonic rudiment (Seidel 1929b, Krause 1934). This peripheral migration, at least in *Platycnemis,* is caused by a contraction wave in the yolk system (see Section VII–B), and hence is passive. In more determinate types of eggs (*e.g.* moth, bee, etc.) the embryonic rudiment arises by differential thickening of the blastoderm, not by cellular migration.

C. Stimulation to Blastoderm-Cell Formation

Seidel (1932) reports that if the activation center (*q. v.*) is eliminated in *Platycnemis* by constricting the egg, there is a delayed formation blastoderm of extra-embryonic type anterior to the constriction. Reith (1931b) reports a somewhat similar state of affairs in *Camponotus,* where if the activation center is eliminated immediately after fertilization the nuclei reach the

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distinct, so too there must be some intrinsic difference between vitellophag and blastoderm nuclei. Since plasma streaming is a proven fact and in at least some cases has a definite relation to blastoderm formation (see Section V–C), we prefer for the present to consider nuclear movement as passive and these vitellophag cases as exceptions to the *general effect* of plasma streaming rather than as absolute refutation of any causal significance of the streaming. Another possibility is that the intrinsic nuclear differences result in local surface tension phenomena at the surface of the various nuclei and so influence the plasma streaming in their own immediate vicinity or the movement of the particular nuclei in this streaming.

surface of the egg but are incapable of blastoderm formation, nevertheless they undergo differentiation into embryonic and extra-embryonic types.

Eastman (1927) reports that in *Pieris* the nuclei enter the cortical layer perpendicularly and move outward through the cortical layer to protrude beyond. It is difficult to say whether this protrusion is due to continued outward movement of the nuclei or to withdrawal of the cytoplasm between them. At this time the cytoplasm of the cortical layer can still be distinguished from that of the protoplasmic islands, and it is from the outer part of the cortical layer that the cell walls begin to develop. By this time the 'tails' of the protoplasmic islands have been drawn in and fused with those of neighboring nuclei to form the inner cortical layer. The cell walls now grow inwards between the nuclei; the basal membrane is then formed through the center of the inner cortical layer dividing it into two thin layers. The walls begin to develop anteriorly where the nuclei first enter the cortical layer, and are completed ventrally before dorsally.

Seiler (1924) reports a case in the eggs of *Phragmatobia* (moth) hybrids that has remained unique among insects. These eggs were presumably unfertilized since no sperm could be found and the egg nucleus, which remained lying at the point where the two nuclei usually fuse, soon disappeared and left only the protoplasmic island to mark its former position. Such eggs frequently ("hundreds") underwent partial or complete division of the cortical layer into blastoderm cells of fairly normal appearance but without nuclei. He found all stages from early division to a complete 'blastula,' and his photos leave no question of the authenticity of his observations. It is of particular interest that the division was not uniform throughout the egg but was quite 'spotty.' The parts of the cortical layer that divided became thickened (as in normal eggs) in contrast to unthickened in undivided parts and in eggs that did not develop. There is, then, a definite correlation in this case between the thickening of the cortical layer and its division into 'cells.'

Schnetter (1934a) reports that the Honey Bee is very similar to *Pieris,* differing principally in that the basal membrane is formed before the formation of the inner cortical layer. It soon

disappears, the inner cortical layer is incorporated into the blastoderm, and then the basal membrane is re-formed.

Experimentally Strasburger (1934) shows that there is a definite time relation between the entrance of the nuclei into the cortical layer and the formation of cell boundaries in *Calliphora*. Normally both occur synchronously throughout the egg, but when the nuclei enter the cortical layer first at the anterior end (either abnormal distribution or experimental hindrance) the cell boundaries also form there first. [This time relation is also seen in cases where the nuclei do not enter the cortical layer synchronously, *e.g.* first reach surface ventrally in *Ephestia* (Sehl 1931), antero-ventrally in *Pieris* (Eastham 1927), etc. In these cases the cell walls form first in those parts where the nuclei first reach the surface.]

From the above data four points are noteworthy: (1) Seidel's and Reith's observations that delay in (*Platycnemis*) or failure of (*Camponotus*) cell-wall formation occurs after elimination of the activation center; (2) Eastham's observation of the formation of cell boundaries from the cytoplasm of the *original* cortical layer; (3) Seiler's observation of 'cell' formation without nuclei but with a thickening of the cortical layer; and (4) Strasburger's demonstration of the formation of cell walls at a definite time after entrance of the nuclei into the cortical layer. The first suggests that cell formation is aided or determined by the activation center; the second might be interpreted as suggesting that there is a predetermination of the cortical layer to form cells; the third must be interpreted as either a partial activation causing both localized streaming and cell formation or as a partial activation causing streaming which in turn causes cell formation; and the fourth suggests that cell formation is wholly a function of the nuclei. The natural assumption is that all these factors are involved but that they have different potencies in different insects.

VI. THE INDETERMINATE–DETERMINATE SERIES

By differences in the time of appearance of the visible morphological characteristics of development, especially the germ

cells, Seidel (1924) showed that it is possible to establish a series ranging from indeterminate through incompletely determinate to determinate types. In indeterminate eggs the visible separation of regions of different prospective significance occurs after blastoderm formation, and organ segregation follows after differentiation of the germ layers and segmentation. In determinate eggs this visible differentiation occurs before or during blastoderm formation.

Following this lead experiments were devised by Seidel and his students to determine if this series is also illustrative of the potencies of the egg parts as determined by the regulative power or conversely the degree of predetermination of the egg parts. These studies have shown that Seidel's series is fundamentally accurate both for visible differentiation and for the degree of predetermination of the various egg parts. From both viewpoints it is a matter of time (time or stage of visible differentiation and time of determination), indeterminate eggs becoming more and more determinate as development proceeds, determinate eggs being fully determined by the time of fertilization. Schnetter gives the following revised scheme:

Indeterminate — Incompletely Determinate — Determinate

Odonata—Hemiptera—Orthoptera—Coleoptera—Hymenoptera—Lepidoptera—Diptera.

Whether or not Schnetter is justified in listing the above sequence seems open to question. In the first place no experimental data are available for either the Hemiptera or the Lepidoptera, and these two orders are placed in the series wholly on a basis of the time of visible differentiation of the various parts. A more serious criticism lies in the implication of homogeneity within single orders. Experimentally only a single species of Odonata has been studied, two Orthoptera, two higher Hymenoptera, three Coleoptera and three higher Diptera. As will be pointed out below there is good reason to expect considerable variation within the order Hymenoptera, polyembryonic forms seeming to be highly 'indeterminate.' Perhaps similar variation will be found within other orders.

Indeterminate eggs have a certain amount of determination at the time of fertilization. Bilaterally symmetrical ones have

the polarity and dorsal and ventral sides irrevocably determined. In the absence of experimental data it is not possible to evaluate the variation in position of the embryo of the radially symmetrical egg of the bug *Pyrrhochoris* (see Section III); though the embryonic axis varies through 90°, it is not possible to say whether its position is determined before, during or after fertilization. Except for the fundamental axes indeterminate eggs possess great regulative powers as shown by dwarf embryos and duplications. Experimentally produced twins and duplicated parts show that there is in the early blastoderm a specific disposition of materials for the formation of the germ band but that when these materials are separated each part tends to form a whole structure rather than only an isolated part. This power becomes more limited as development proceeds, but studies on regeneration show that the power of regulation is not entirely lost until the adult stage (see Part II).

In *Platycnemis* Seidel (1928, 1929a) reports that shortening the longitudinal axis by constricting the egg results in dwarf but otherwise normal embryos. Duplications which result from splits induced by cautery also show that the prospective potency of the egg parts is greater than the prospective significance at the time the various anlagen are set apart; each duplicated part is larger than one-half the size of the same organ in a normal embryo, so that the sum of the two duplicated parts is greater than the size of a single normal organ. This power of duplication is possessed by all organs, external and internal. It never occurs in the longitudinal axis, seldom in the dorso-ventral axis, but commonly in the transverse axis. The interpretation given is that determination occurs in these three axes in this same order. In *Tachycines* Krause (1934) reports a correlation between the type of mechanical injury to the germ band and the effect. Frontal fissures give duplications in the dorso-ventral axis, median and oblique fissures of various extents and positions give symmetrical or asymmetrical, transverse anterior or posterior duplications or parallel twins (division of differentiation center ?), and fine splits give duplications of single organs. Effects produced by operating at different stages show that determination of the main axes is followed by determination of segments

in the longitudinal axis, then of the lateral halves within these segments, and finally of individual organs. Oka (1934) reports preliminary experiments showing only that the egg of the cricket is of the regulative type.

An interesting observation is made by Cappe de Baillon (1925, 1927). Because he has shown that "double monsters" of phasmids (Orthoptera) most probably originate from the fusion of two eggs, he suggests that the oöcytes were partially determined before fusion so that the determined parts of the cortical layer did not fuse completely into a single embryo. He reports that the chance of any given organ being double is correlated with its nearness to the procephalic lobes—the more anterior the more frequently double. This suggests to us that the determination process in the cortical layer of the growing oöcyte begins at or near the anterior end and proceeds posteriorly, the great regulative power of the indeterminate egg permitting fusion of the less determined regulative power of the indeterminate egg permitting fusion of the less determined parts (posterior regions) but not of the more determined parts (anterior regions). Naturally the reverse is true for the experimental production of twins and duplications. An alternative view would consider this the result of the incomplete or dissimilarly oriented fusion of two differentiation centers (Schleip 1929).

Slifer (1934) shows that dechorionated grasshopper embryos can withstand a lowering or 10 per cent or raising of 30 per cent of the osmotic pressure without noticeable effect. A 0.3n Ringer's solution has about the same severity of effect as a 3n Ringer's, but embryos will survive many hours in both and in even more anisotonic solutions. Within certain limits the changes are quickly reversible on return to normal Ringer's. Comparison with *Drosophila* suggests that this may be another criterion of indeterminacy.

Incompletely determinate eggs are intermediate between the two extreme types. They are capable of considerable regulation following experimental interference in early stages, but the power is lost much sooner than in indeterminate eggs. Dwarf-formation has been observed but not duplication of parts. The absence of duplications indicates that strict determination of the presumptive anlagen occurs early.

In the Hymenoptera Schnetter (1934b) reports that constricting the Honey Bee egg at the stage of the "uniform blastoderm" (12 hour embryo) can result in a normal dwarf embryo. Later blastoderm stages (24 hour embryo) are less labile and more inclined to mosaic formation with a shift in the regions of different prospective significance (Fig. 2). In the ant *Camponotus* Reith (1931b, 1932b) shows by cauterization and constriction that the egg possesses considerable regulative power until the completion of the visible zonation by differential thickening of the cortical layer (presumably under influence of the activation center). After that it is determinate though not as highly so as dipterous eggs. By centrifuging he shows that stratification of the egg parts prior to the cortical zonation blocks development but later centrifuging does not prevent formation of a fairly normal germ band. These two sets of data show that within this order there is a difference in the time of determination, that of the ant occurring noticeably earlier than that of the Honey Bee. Though there are no experimental data involved, polyembryony (the production of two or more, sometimes several hundred, embryos from a single ovum by division of the cleavage cells into groups) in parasitic Hymenoptera must be viewed as positive evidence of a high degree of regulative power.

In the beetle *Bruchus* Brauer & Taylor (1934) show by constriction and cauterization that determination occurs quite early, the egg becoming a true mosaic during blastoderm formation. In *Sitona* Reith (1935) reports that the determination process occurs slightly later than in the ant and seemingly at about the same time as in the Honey Bee. In Chrysomelid beetles Hegner (1908, 1909, 1911) performed a series of experiments using puncturing, cauterization and centrifugation but his results seem open to some question, partly due to the extreme nature of injury inflicted upon the egg. He claims that when the germ-tract determinant region or the 'pole cells' are removed or killed no germ cells are found in the larva (this seems probable, see Section X), and that the egg shows no regulation during cleavage, blastoderm or later stages, *i.e.,* that it is strictly mosaic from the time of laying. Some of the results of centrifuging these eggs resemble those obtained with *Camponotus,* normal embryos (sometimes displaced) de-

veloping only in eggs centrifuged during the blastoderm stage or later, though one centrifuged female *Leptinotarsa* laid stratified eggs which developed normally. In all these beetles the investigators agree in saying the eggs are completely determined (mosaic) after the formation of the blastoderm.

Determinate eggs have been found only in the Diptera but at least as far as the germ cells are concerned Hegner's results indicate that the Chrysomelid beetles might also be placed here (and also the other insects in which the presumptive germ cells are segregated as 'pole cells' during blastoderm formation). Determinate eggs always give mosaic formations under experimental conditions, the few slight exceptions showing an extremely small amount of regulative power. Accordingly the parts of the embryo must be looked on as entirely self-differentiating after fertilization, or, at least, incapable of development beyond their prospective significance.

Reith (1925), Pauli (1927) and Howland & Robertson (1934) report that cauterization of dipterous eggs invariably gives mosaic development. Slight regulative power is shown by Reith's report that the midgut anlagen of either end may form an entire midgut, and also by Strasburger's (1934) report that slight cauterizations at the beginning of cleavage may delay the migration of the cleavage nuclei to the posterior part of the egg without preventing formation of a normal embryo—the posterior parts developing slightly later than the anterior parts. Sonnenblick (1934) and Howland & Child (1935) show that puncturing does not necessarily prevent the development of normal embryos and larvæ although the adults may exhibit injuries. The high mortality and uncertainty as to the exact nature of the extruded materials make their data difficult to interpret under this section. On a basis of ultra-violet irradiation of oriented *Drosophila* eggs Geigy (1931b) postulates two determination periods in the eggs of higher Diptera: (1) an embryonic determination process completed not later than the time of fertilization, and (2) an adult determination process *via* the presumptive imaginal discs in later embryonic life. This will be discussed later.

Howland & Robertson (1934) report that dechorionated *Drosophila* eggs develop normally in "sea water diluted with tap

water to 33 per cent.'' They do not mention the effects of other solutions or concentrations but Howland (1932) reported that *Drosophila* sperm are highly sensitive to the constitution of the medium. The solution used for eggs is the same as that she found most satisfactory for sperm.

Cytoplasmic determination: It is evident that the distinction between 'regulative' and 'mosaic' eggs in insects, as in other groups, is not sharp, and the designations mean little unless the period of development is specified. The earlier an egg assumes a mosaic nature the 'more determinate' it is, but even the most 'indeterminate' of insect eggs eventually becomes 'determinate.' Sections V and VI show also that the primary seat of the chemodifferentiation (predetermination) is the cytoplasm as a whole, and that the formation of cells plays only a secondary or indirect part. The fact that development is less disturbed by late than by early centrifuging (Hegner 1909, Pauli 1927, Reith 1932b) suggests that fixation of determination is accompanied by increased cytoplasmic rigidity (viscosity) either before or after cell-formation.

VII. DEVELOPMENTAL CENTERS

A. The Activation Center

Seidel (1929b) has used the term ''Bildungszentrum'' to designate a region situated near the posterior pole of the egg and necessary for development. As we shall show this center is responsible for the activation of the egg system, and so it is here designed 'the activation center.'[6] The literal translation, 'formative center,' is ambiguous and may carry unwarranted implications. It is not a visibly distinct part of the egg but can be more or less closely delimited by experiments. It functions by the production of some substance which diffuses forward through the interior of the egg and initiates development. In its absence no true differentiation of the embryo occurs. To date it has been demonstrated in five species of insects (a damsel fly, two ants and two beetles).

The activation center has been most elaborately analyzed in the damsel fly *Platycnemis* by Seidel (1926–1934). In this species (Fig. 1) the anterior border of the activation center coincides

[6] This term has already been used by Huxley & DeBeer (1934).

approximately with the presumptive posterior end of the embryo (about 1/9th the length of the egg from the posterior pole) but its posterior extent is undetermined. The point at which the germ band later invaginates into the yolk marks its locus (see Section VIII). Here the functioning of the activation center depends upon an interaction between the cleavage nuclei and the region of this center. This is demonstrated by numerous experiments:

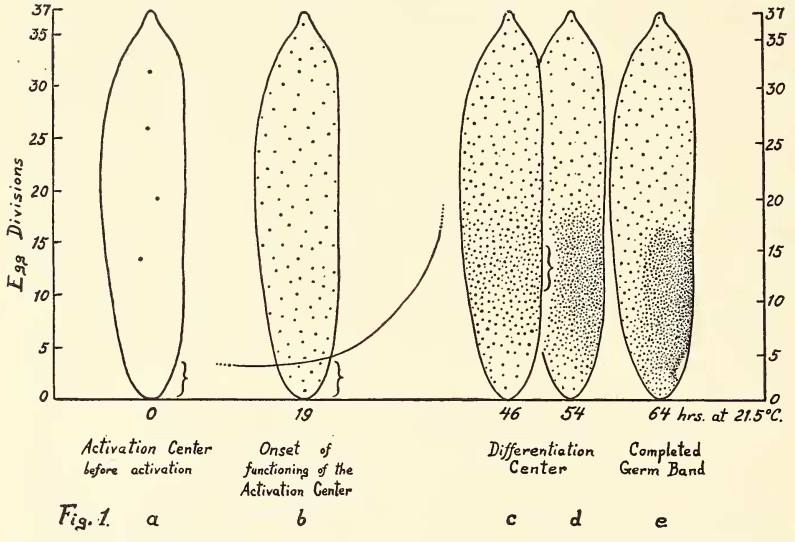

Figure 1. Graphic diagram of the development of the egg of the damsel fly *Platycnemis*. *a* 4-nuclei stage with bracket indicating the position of the activation center. *b* 256-nuclei stage with curved line indicating the diffusion anteriorly of the product of the activation center. *c* Beginning of cell-aggregation with bracket indicating the position of the differentiation center. *d–e* Formation and completion of the embryo anlage. 1 egg division equals 24 μ. (After Seidel, 1934.)

when the cleavage nuclei are late in reaching this region either due to abnormal distribution, killing of one of the first two cleavage nuclei with ultra-violet light, or constricting the egg and later removing the constriction, the functioning of this center is delayed. Careful comparison with normals shows that none of these delays causes any effect on later development other than delaying the onset. If, however, the cleavage nuclei are prevented from entering this region by a constriction of the egg no

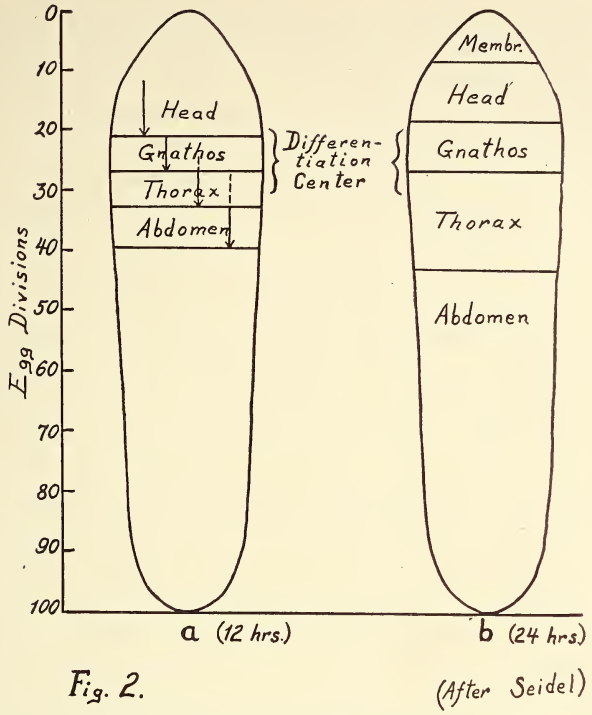

Figure 2. Schematic anlagen plan of the Honey Bee egg. *a* Posterior boundaries of the *potency regions* ("... those regions of which a small part must remain in the egg to enable the formation of the entire corresponding organ region") in the 12-hour blastoderm. *b* Expansion of the same regions along the longitudinal axis in the 24-hour blastoderm. (After Schnetter, 1934b.)

development ensues although the anterior parts continue to live, undergo 'yolk cleavage' and eventually form an extra-embryonic blastoderm. Partial constrictions show clearly that it is the nuclei which must reach this area, not some chemical which can diffuse through the yolk system. The reaction is not limited to the surface of the egg (which lacks a distinct cortical layer) since peripheral parts in the region of the center may be killed by ultra-violet light without preventing normal development. Finally, the removal of constrictions and constricting after the nuclei enter this region both show by the production of embryos that the injury to the egg does not block development. There-

fore we must conclude (1) that the egg contains two quantitatively or qualitatively different regions (compare animal and vegetal poles of other eggs), and (2) that neither the cleavage nuclei and their cytoplasm nor the activation center is capable of producing a normal or even partial embryo alone, but that they react together to furnish a product which allows normal development to proceed along axial and symmetrical lines that have already been laid down.

Seidel further shows that after the nuclei have entered this region, more and more can be constricted off from the posterior end of the egg without blocking development. The action of the center spreads slowly over a considerable area so that well before the assembling of cells to form the germ band its effect has been felt over the entire presumptive germ band (see curved line in Fig. 1). The interaction product must diffuse forward through the yolk system since a partial constriction after the nuclei reach the activation center does not prevent an embryo being formed anterior to the constriction. In some eggs there is a visible change in the structure of the yolk proceeding apace with the diffusion of factors from the posterior pole anteriorly. Further, this product activates the differentiation center which will be discussed below.

In the ant *Camponotus* Reith (1931, 1932) shows by constriction and cauterization that an activation center is present near the posterior pole but he has not delimited it closely. In this species it differs from *Platycnemis* in that the cleavage nuclei are not responsible or even necessary for its functioning. Its activation must be induced by some product concerned in fertilization, presumably produced not later than the time of fusion of the egg and sperm nucleus since the visible effect of the functioning of the activation center is seen in very early cleavage stages. This product must diffuse through the yolk system just as the product of the activation center does since neither is impeded by partial constrictions, yet obviously they cannot be the same substance. Another interesting difference from *Platycnemis* is that the activation center in this species induces a visible zonation (differential thickening) of the cortical layer prior to the migration of the cleavage nuclei. This visible differentiation begins at

the posterior pole and passes slowly to the anterior end of the egg as does also the 'activating power.' Attempting to localize the activation center to some visibly distinct part of the posterior region of the egg he noted that the disintegration of the 'pole-disc' is correlated with the action of this center. The 'pole-disc,' however, can not be the essence of the center since it is lacking in the ant *Lasius* which gives similar experimental results. Nor can the effect be traced to the bacterial symbionts since their injury is not correlated with that of the embryo. [It is interesting to note that Hinman (1932) shows various bacteria present in a small percentage of mosquito eggs without seeming effect on the embryos, and Scheinert (1933) discusses their presence and perpetuation in various insects.] As in *Platycnemis,* the activation center must be ascribed to an invisible difference in the egg regions.

In the beetles *Bruchus* and *Sitona* Brauer & Taylor (1934) and Reith (1935) report the presence of an activation center in the posterior region of the egg. In both of these beetles the functioning of this center begins during cleavage and is finished when the nuclei enter the cortical layer (*i.e.,* later than in ants). Unfortunately these are both rather preliminary reports and analyses must be made with care. In *Bruchus* Brauer & Taylor report that when the constriction is made sufficiently early to exclude nuclei from the posterior end of the egg, the anterior portion forms "only a poorly developed blastoderm," yet "a protoplasmic isthmus . . ., however narrow it may be, serves to conduct the organizing principle anteriorly." Hence it would seem that the nuclei are necessary for the functioning of the activation center in *Bruchus* as in *Platycnemis.*

B. The Differentiation Center

The visible differentiation of the insect embryo begins in the presumptive prothorax, and from this region proceeds anteriorly and posteriorly. Descriptive embryology shows that the spread of differentiation from the thoracic region is a general principle of insect development,[7] and, as Schnetter (1934a) points out, may

[7] Schnetter (1934a, table 12) gives a comparative table including representatives of all the major orders.

be considered as typical of insects as is superficial cleavage, though there may be exceptions in regard to both (in other arthropods and annelids, differentiation begins in the head). There is evidence that this also applies to pupal development. Seidel speaks of "das morphologische Differenzierungszentrum" and "das physiologische Differenzierungszentrum," but since these two terms denote distinct concepts it is deemed preferable in this paper to avoid this terminology, although, in general, Seidel's treatment is followed. The term 'differentiation center' is here restricted to a physiological concept and is not applied to the visible starting point of differentiation, even though physiological and morphological phenomena normally occurring at a common locus may at times appear to be experimentally separable.

In *Platycnemis* the region where the two rows of assembled nuclei first come together to form the germ band marks the place from which all later differentiation proceeds anteriorly and posteriorly; it lies in the presumptive thoracic region, about one-third the length of the egg from the posterior pole (Seidel 1926, 1929b, 1934). In the Honey Bee (Schnetter 1934a) the visible embryonic differentiation begins near the anterior end of the egg (presumptive cervical region) at the site of polar-body formation, where the cortical layer is thickest, reticular cytoplasm is most concentrated, cleavage nuclei become most numerous and first reach the surface,. vitellophags first appear, heterochronous mitoses first set in, and cell-partitions are first formed. A qualitative distinction of this region during cleavage is indicated by differential staining with thionin but not with haematoxylins. This region also has precedence in all later differentiation (formation and closure of mesodermal furrows, segmentation, appearance of appendages, etc.). A similar region is evident in *Ephestia* (Sehl 1931) but here the proctodæum forms before the stomodæum, 'yolk cleavage' progresses from posterior to anterior, and dorsal closure of the body wall is completely last on the meso-metathoracic boundary. Eastham (1927) describes the differentiation processes in *Pieris* as occurring "from before backwards." Spread of morphological differentiation from a definite region is not so evident in the eggs of higher Diptera.

Experimental methods have demonstrated, as one might expect, that this region of initial morphological differentiation is of fun-

damental physiological significance, incorporating the so-called 'differentiation center.' The results of constricting *Platycnemis* eggs (Seidel 1934) show that this center normally coincides with the starting point of morphological differentiation though seemingly this morphological manifestation can be experimentally separated from the primary physiological center. The differentiation center extends from the second gnathal (maxillary) segment to the second thoracic segment with its midpoint in the anterior half of the presumptive prothorax (Fig. 1c, 4a).

Seidel (1929b, 1931, 1934) further shows that no differentiation can occur in an isolated region of the egg of *Platycnemis* unless the differentiation center is present in whole or in part. The center can function only after the product of the activation center has reached it by diffusion. It does not affect blastoderm formation but does directly influence the assembling of blastoderm nuclei and heterochronous divisions in germ band formation, although heterochronous divisions alone occur when almost the entire center is tied off. Unlike the activation center, the differentiation center cannot function normally with even a relatively slight constriction of this region of the egg. A very loose ligature outside the boundaries of this center will allow the formation of the germ band on both sides of the ligature, but if it is only slightly tighter, even though the blastoderm and yolk remain unsevered, the germ band forms on one side only. Since no continuity is destroyed in any part of the egg by such a constriction, the center's action cannot depend solely upon the spread of a substance but must involve an energy transfer, *i.e.*, must be a dynamic phenomenon. Cell aggregation to form the germ band is not prevented by killing a complete girdle of blastoderm cells over the entire region of the center (by ultra-violet irradiation). Initiation of this process must therefore originate in the 'yolk system,' viz., the yolk with its included cytoplasmic reticulum and vitellophags. Localized contractions of the surface of the yolk system, produced by cautery or point-irradiation before or during the time of cell aggregation, result in changes in the length, shape and position of the embryonic rudiment. The spaces between the yolk and chorion thus formed, or resulting when a ligature is loosened, serve as foci for cell aggregation which can thus be

artificially and prematurely produced outside the normal region. These data indicate that the direct action of the differentiation center is due to a wave of contraction in the yolk system spreading anteriorly and posteriorly from the center, the yolk system retracting from the chorion in such a way that the evenly distributed blastoderm cells are forced to fill the resulting space. In this manner cells first aggregate in the region of the differentiation center, and the size and shape of the germ band is molded by the space between yolk and chorion. This makes it clear how the differentiation center can be the center of a field of activity without necessarily involving the actual transport of any material substance, the result of constructions being due to interference with yolk movements. Additional proof of the dynamic nature of the differentiation processes in *Platycnemis* is furnished by the fact that killing a girdle of cells by ultra-violet irradiation either in front of or behind this region where visible differentiation normally begins causes a corresponding shift (backwards or forwards respectively) of the position of the initial differentiation. The yolk system is in effect temporarily reduced in size and the visible differentiation begins in the same relative position within the new system that it held in the larger system. If, then, the differentiation center is a definite region within the egg (as it seems to be), it must remain at one end of the reduced system while its action originates in a new location.

In the Camel Cricket *Tachycines* (Krause 1934) the order of decreasing regulation ability (lost earliest by the thorax), the relative frequency of organ-duplications (least often in prothorax), and certain asymmetrical duplications show that differentiation along both longitudinal and transverse axes proceeds anteriorly and posteriorly from the first thoracic segment. He suggests that the presence of a differentiation center may account for the non-occurrence of doubling along the longitudinal axis.

The results of constricting eggs at successive points along their length enabled Schnetter (1934b) to mark off 'potency regions' in the 12-hour blastoderm of the Honey Bee egg. Each of these regions when present in whole or in part guarantees the *complete formation* of the corresponding embryonic systems (*cf.* 'harmonic equipotential regions,' *e.g.,* limb-field in amphibia). Thus regu-

lation in the Honey Bee egg is, in general, 'stepwise,' a given region developing as a whole or not at all. In fig. 2a it can be seen that all the potency regions lie within or extend into the region of the differentiation center. Accordingly it may be regarded as a "concentration center" for potencies enabling whole-formation of the various organ regions. Fig. 2b shows that there is a shift of these potency regions during the time between the 12- and 24-hour blastoderm. This, with a few preliminary experiments on an intermediate stage, indicates that there is a gradual shifting of these boundaries out of the differentiation center and into their definitive position. These facts suggest that the differentiation center bears a causal relation to the development of the embryonic regions. The fact that a fixed (morphological) region of the bee egg may have a prospective potency in an early stage that is entirely different from (not merely more extensive than) its prospective significance as shown in a later stage, indicates that chemo-differentiation is not only progressively increasing as development proceeds but that there is also occurring a *redistribution* of the chemo-differentiated materials. Further a shift in the region of first visible differentiation in developing dwarfs following constrictions in the 12-hour stage (as evidenced by the more posterior position of the beginning of the mesodermal furrows) indicates that this point assumes about the same relative position in the decreased whole as in a normal egg. So presumably dynamic factors are of fundamental importance in the egg of the Honey Bee as well as *Platycnemis*.

In the ant *Camponotus* Reith (1931b) noticed that defects resulting from late cauterization of the presumptive anlage-region suggested a dependence of the posterior regions upon the anterior for normal differentiation. He reports two cases in which defects at the anterior end of the germ band resulted in the absence of differentiation although the nuclei migrated normally. Presumably these represent an elimination of the differentiation center.

In the beetle *Sitona* Reith (1935) reports admittedly incomplete experiments indicating the presence of a differentiation center located in the central region of the egg. This center seems to be stimulated by the activation center since no development occurs when the latter is destroyed during cleavage. He says that by

analogy with *Platycnemis* and the Honey Bee this center must be assigned a "regulative" significance.

In the higher Diptera the data from cauterization, constriction and centrifugation (Reith 1925, Pauli 1927, Rostand 1927, Strasburger 1934) show that in all cases mosaic or partial embryos are produced by elimination of any egg part irrespective of the age of the embryo. A physiological differentiation center affecting larval organization is not demonstrable although Henshaw's data (1934) indicate that there is some physiological regulator of early embryonic differentiation in *Drosophila* since only weak doses of x-rays were required to block development before gastrulation but much stronger doses were required later. Also, Geigy reports (1931b) that in the production of defective adults of *Drosophila* by ultra-violet irradiation of eggs, both the sensitization and desensitization to the rays begins in the thorax and proceeds posteriorly as a function of age. This is seemingly to be interpreted as a differentiation center in the presumptive adult thorax, the effect of which passes progressively posteriorly, even though no such center has been demonstrated for larval organization.

C. Interaction of Centers and Other Regions of the Egg

Seidel (1934) has shown that in *Platycnemis* an interaction between the cleavage nuclei and the region called the activation center results in a product which diffuses forward in the egg (see Fig. 1 a–b). As this product passes anteriorly there is a visible change in the structure of the yolk proceding space with the diffusion. However, this product does not directly affect differentiation; it functions by the activation of the differentiation center. The latter, in turn, appears to induce the onset of heterochronous cell divisions and the contraction of the yolk system which brings about the aggregation of cells to form the germ band and the later shift in the position of the same.

Comparison of the reactions of the activation center and differentiation center of *Platycnemis* under similar restrictions indicates that they differ in mode of action. A loose or temporary constriction in the region of the activation center results in no abnormality but only delay. But a loose or temporary constriction in the region of the differentiation center (after functioning

of the activation center) invariably results in an abnormal embryo. This is elucidated by proof that the first functions by the production and diffusion of a specific material substance, the second by dynamic movement processes which must have some as yet unknown physico-chemical basis.

The realization of the importance of dynamic phenomena in development has enabled further analysis of the processes underlying determination and regulation and of the significance of the 'centers.' Such an analysis, based primarily on his findings in *Platycnemis,* has been begun by Seidel (1934). The predominant notes in his discussion are the importance of the entire egg as a substrate for dynamic processes in determination, the subordination of the so-called 'centers' to the system as a whole, and the alternation of dynamic processes and "material reactions" during development. The latter is quite evident in the sequence of events in *Platycnemis,* viz., the migration of cleavage nuclei, the reaction between nuclear and cytoplasmic factors in the activation center, the diffusion of the reaction products cephalad from the activation center, reaction with the yolk system, and the contraction of the yolk system originating in the region of the differentiation center. The determination process is carried out by this alternating series of dynamic processes and material reactions, the former involving the egg system as a whole and enabling the reactions of more or less delimited substances and structures, the centers, to take place. In this light, regulation is not dependent upon the powers of definite centers but upon dynamic processes or structures made possible through such processes. It follows that regulative ability is limited by the degree of rigidity of the arrangement of all the substances and structures necessary for development. Regulation can occur only in that part of the egg in which normal dynamic processes can transpire, and the degree of regulation depends upon the extent to which they can proceed unhampered. As Seidel says, the entire egg must be regarded as a system in which not only the embryonic tissues and included factors but also the extra-embryonic parts must be held responsible for the determination of the organ-regions.

In no other group is there a set of data for any one species sufficiently extensive to permit such a complete analysis of factor

interaction. Reith (1931, 1932, 1935) shows an activation center at the posterior end of the egg of the ant and weevil. The action of its product is similar to that in the damsel fly but the onset of its action is not dependent on the entrance of cleavage nuclei into the region but is initiated presumably by some part or product of fertilization. However, in the beetle *Bruchus* Brauer & Taylor (1934) give a brief report indicating that the cleavage nuclei are necessary for the action of the activation center. Schnetter (1934b) was unable to demonstrate an activation center in the Honey Bee egg but he has shown the presence of a differentiation center which seems to bear a causal relation to the development of the embryonic rudiment. These data, while differing in certain details from the damsel fly, indicate that dynamic factors are responsible for the arrangement of the structural elements, since regulation involves a shift in the site of initial differentiation along with that of the potency regions. The more rigid the system, the less the regulative power—a principle which may involve the viscosity of the cytoplasm as suggested by the results of centrifuging the eggs of other insects (Section VI). In these insects, also, determination is probably brought about by a harmonious series of interacting processes.

In the determinate type of egg (Reith 1925, Pauli 1927, Sturtevant 1929) we must assume that the determination attained by such series of events is in large measure completed by the time of fertilization so that the various egg regions are 'self-differentiating.'

D. Comparison of Insects and Amphibia

In drawing a general comparison between insect and amphibian development, Seidel (1934) regards both the insectan differentiation center and the amphibian organizer as "factor regions" of which the ability to function is subordinate to the dynamic processes of the entire egg. In both, regulation is effected not through the developmental centers but through the system as a whole which the centers subserve. Fundamentally the determination processes in the two groups may be similar, but he adds that more detailed comparison must await further analysis of the causal relation of the insectan differentiation center to organ for-

mation. Evidence from the Honey Bee (Schnetter 1934b) indicates a similarity between the differentiation center and the organizer, in that the former is a concentration center for potencies enabling regional differentiation along the entire length of the egg, and the latter seems also to possess individuation factors.

Seidel also compares the action of the insectan activation center with the action of the inducing substance of the amphibian organizer. In *Platycnemis,* after the cleavage nuclei migrate into and react with the activation center, a substance diffuses forward through the yolk. In Amphibia, the organizer region (chorda-mesoderm) is carried under the ectoderm by the process of gastrulation, and the inducing substance reaches the overlying layer by direct contact. Interaction in the sense of an underlying layer influencing an overlying layer has not been demonstrated in insects.[8] In both insects and amphibia dynamic processes are instrumental in bringing the inducing substance to its field of action where it is the precursor of further dynamic processes—contraction of the yolk system in the one case, formation of the medullary tube in the other.

We have omitted two points from Seidel's comparison. First, his statement that any purely chemical hypothesis of the primary action of the differentiation center is improbable. This is based on the observation that aggregation to form the germ band depends on a contraction wave in the yolk system, and any constriction which interferes with this contraction results in an abnormal embryo. The nature of the origin of this contraction wave is unknown, and in the light of recent work on the chemical nature of the amphibian organizer it seems advisable to leave this question open, though, as Weiss (1935) points out, the 'chemical organizer' is really only an activator, not an organizer. Second, Seidel says that the principal difference between the amphibian organizer and the insectan differentiation center is that the former lays down a dorsal anlage, the latter a ventral anlage. We wonder if this is not as fundamental a similarity as difference since, in both, the embryo forms on that side of the egg which will give rise to the nervous system.

[8] Although metamerism is usually first evident in the mesoderm, there are several cases reported (*e.g., Pieris,* Eastham, 1927) in which it is visible in the ectoderm before in the mesoderm.

Aside from Seidel's discussion, it may be noted that the activation center of the insect egg is similar to the amphibian organizer in (1) its location, (2) its activation in some species by fertilization or some concurrent phenomenon (*e. g. Camponotus*) although in certain species (*e. g. Platycnemis*) the cleavage nuclei are necessary for its functioning, (3) its functioning by the production of a specific substance, and (4) this substance in turn activating or establishing a second center (the differentiation center) which then directs development by dynamic phenomena. There is a paucity of data regarding causal relationships of the developmental centers to organ formation but this is also Seidel's principal reason for refraining from a definite analogy af the insectan differentiation center with the amphibian organizer.[9] The comparison leads inevitably to the question as to whether the differentiation center is to be considered a primary developmental center which is merely activated by the product of the activation center, or a secondary center produced by the activation center or by factors involving the egg as a whole. Seidel favors the former interpretation, but from the available data it seems that the latter is possible. Some of the observed effects of reducing the yolk system by partially constricting the egg of *Platycnemis* could also be explained by postulating that there is a certain minimum size to which the system can be reduced before it becomes incapable of producing the contraction wave leading to germband formation. Tying off the original locus of the differentiation center may be simply reducing the system so much that the forces concerned cannot bring about the simulation of the normal visible processes. In this light the differentiation center would be a focal point of forces—an effect, rather than a fixed region of causal factors. However, the production of partial embryos by complete constriction is possible evidence that the differentiation center may be a fixed region of potency factors. It seems that the exact nature of the differentiation center can be decided only by experiments analogous to the extirpation and explantation of organizer material in Amphibia. In this connection Weiss (1935) makes the illuminating suggestion that the activating and

[9] The only insect in which any causal relationship of the differentiation center to organ formation is known (Honey Bee) is one in which we know nothing about the activation center.

organizing functions which are combined in normal amphibian development, are spatially separated into two centers, the activation center and the differentiation center, in normal insect development.

VIII. BLASTOKINESIS OR MOVEMENTS OF THE EMBRYO

Prior to the differentiation of striated muscle fibers the insect embryo frequently undergoes extensive movements. These movements vary greatly in different insect groups (see Imms 1934 or Snodgrass 1935). They have been experimentally studied in the grasshopper *Melanoplus*. Here blastokinesis consists of a reversing of the longitudinal axis followed by a revolution around this axis. Slifer (1932a) shows that this change of position is accomplished by vigorous movements of the embryo itself. These movements originate as contraction waves running along the lateral borders of the dorsally-incomplete abdomen and passing rapidly to the head. With the closure of the dorsal wall and the formation of the dorsal vessel they seem to become resolved into the heart beat as was suggested by Nelsen (1931). In overwintering eggs, diapause interrupts incipient blastokinesis as well as an other developmental activities, these processes being resumed immediately after the end of the diapause period (diapause will be treated in Part II).

Although the embryonic membranes are usually ruptured Slifer reports one positive case in which blastokinesis was initiated and partly completed without the rupture of the serosa, showing that the contraction of the embryonic membranes cannot be the primary cause of revolution. Hence, in the grasshopper, the revolution of the embryo must be due to its own movements. In sections of embryos of this age she (1934) found unicellular, non-striated, spindle-shaped fibers in the position of the future abdominal muscles. She suggests that these cause the movements (striated muscles do not appear until nine days later).

Concerning the necessity of revolution Slifer (1932a) reports four cases in which it failed to occur and yet the embryos developed more or less normally but were incapable of hatching. But Tirelli (1931) reports that the occasional failure of blastokinesis

in the Silk Worm egg invariably results in death. However, in the latter case blastokinesis brings the dorsal surface of the embryo into a spatially and mechanically more favorable position whereas no apparent advantage is attained in the grasshopper. In *Platycnemis* Seidel (1929b) reports that prevention of blastokinesis by constriction seemingly inhibits development of a posterior part embryo beyond the differentiation of the organ systems, while if the anterior part of the embryo develops in front of an incomplete constriction, blastokinesis occurs in that part of the egg and histological differentiation is completed. Accordingly it seems that blastokinesis is prerequisite for the completion of development in *Platycnemis.*

Although not usually classed under blastokinesis we include here the report of Child & Howland (1933) that the migratiion of the germ cells of *Drosophila* from the posterior pole of the egg to the dorsal surface and thence to the interior of the embryo seems due to the force exerted by the rapid upward growth of the ventral blastoderm. They add that the subject needs further study.

IX. THE ANLAGEN PLAN OF THE EMBRYO

The only satisfactory worked-out anlagen plan of any indeterminate or incompletely determinate type of insect egg is that given by Seidel (1935) for *Platycnemis.* This is well shown in Fig. 3 which gives the blastoderm plan from all three views, and in Fig. 4 which shows the changes undergone during the formation of the embryo. The first phase of shortening of the embryo occurs simultaneously with the onset of action of the differentiation center (Fig. 4 a–b). During this time the presumptive head and abdominal regions contract while the thoracic region increases in length as a result of two movement-tendencies, one drawing the materials towards the region of the differentiation center (between the gnathal and thoracic anlagen), the other drawing the entire presumptive embryo towards the posterior end of the egg. The two tendencies coincide in front of the differentiation center but are opposed posterior to it. In the second phase of shortening (Fig. 4 b–c) the head and gnathal anlagen expand while the thorax and abdomen shorten. During this

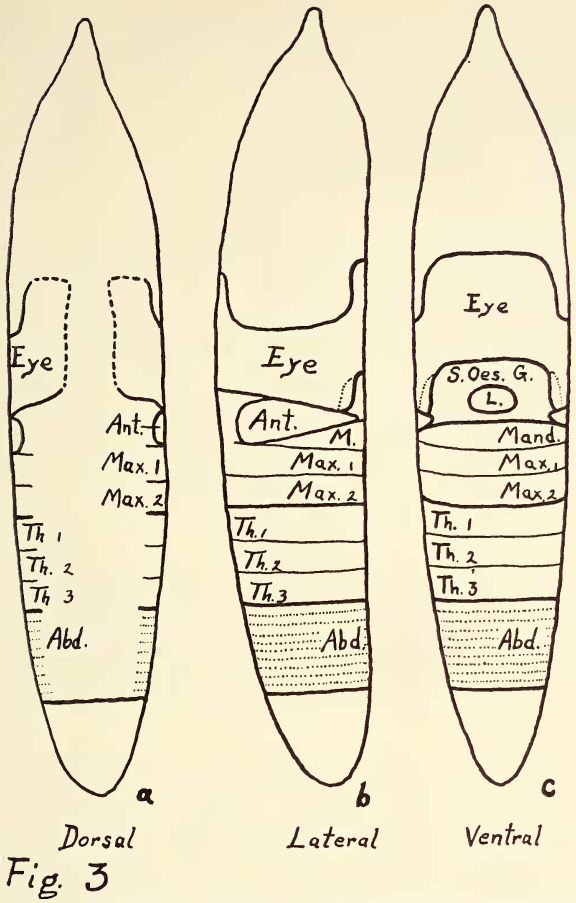

Fig. 3

Figure 3. Plan of the presumptive organ anlagen for the blastoderm stage. *a* dorsal side of egg, *b* left side, *c* ventral side. The numbers indicate the divisions of the egg (1 division equals 24 μ). *Abd* abdomen anlage, *Ant* antenna anlage, *Eye* Eye anlage, *L* labrum anlage, *Mand* mandible anlage, *Max 1–2* maxillae anlagen, *Th 1–3* thoracic segment anlagen, *S.Oes.G.* Supraoesophageal ganglion anlage. (After Seidel, 1935.)

phase the action of the differentiation center is no longer apparent. The entire embryo continues to move posteriorly and soon invaginates into the yolk. During and immediately following invagination the parts of the embryo elongate and assume larval proportions.

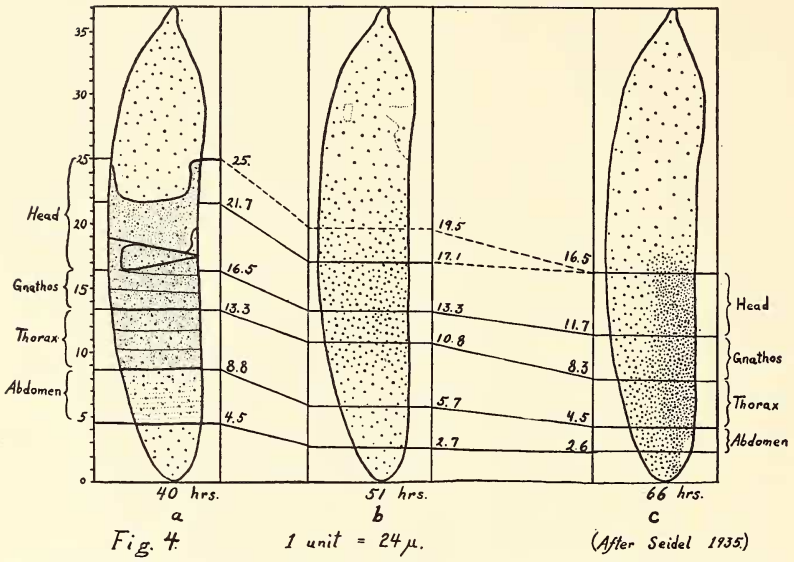

Figure 4. Graphic representation of the shifting of the anlage regions of the left side of the egg in reference to the movement of cell-nuclei. *a* Blastoderm stage through *b*, cell aggregation to form the germ band, to *c*, the completed embryo anlage. Abscissa: Time in hours after the 2-nuclei stage. Ordinate: Number of divisions from the posterior pole of the egg (1 division equals 24 μ). (After Seidel, 1935.)

From Seidel's detailed discussion of irradiation defects as marks in making these maps we note the following: (1) The study is hindered by regulation processes. (2) Raising the temperature accelerates the differentiation process more than the healing process and so aids the formation of defects. (3) For certain organs the experiments show a 'defect correlation' rather than a 'developmental correlation.' For instance there is a high correlation of eye and thoracic defects which Seidel suggests may be due to a primary defect changing yolk contraction and thereby altering the molding of the embryonic anlage. (4) Defect experiments do not show the true morphological course of development owing to the indirectness of physiological investigation; more truly they indicate a plan of the various factor regions.

Schnetter (1934b) gives a partial segmental map of the 12- and 24-hour blastoderm of the Honey Bee egg (Fig. 2) showing

a shift in the prospective significance of the parts of the blastoderm between these two stages. Incidentally he says that after the formation of the germ band the differentiation center no longer belongs to the structure of the whole egg but only to the embryo. With the shift of the presumptive embryonic parts the middle of the differentiation center shifts from division 24 of the egg to division 28.

For the determinate (mosaic) egg of the Diptera Reith (1925) and Pauli (1927) show that the parts of the embryo originate as presumptive anlagen at the same points where they later make their appearance, and that little or no regulation occurs. Sonnenblick (1934) and Howland & Child (1935) report that normal larvæ and adults may develop from punctured *Drosophila* eggs from which a portion of the contents has been extruded. Due to doubt as to the exact nature of this extruded material it is not possible to evaluate these results. However, Sturtevant (1929) shows by genetic analysis of *Drosophila* gynandromorphs that the presumptive imaginal discs must occupy the same relative positions in the blastoderm as the points where they later make their appearance in the larva. As healing processes are not involved in this case it seems that a shift in prospective significances such as Schnetter describes for the Honey Bee does not occur in *Drosophila.*

X. ORGAN FORMATION

1. **Endoderm:** There are no really pertinent experimental data. The midgut is clearly not the primitive archenteron. It is formed, practically regenerated, later in development from rudiments. Eastham (1927) and Snodgrass (1935) review the subject from a comparative-morphological standpoint. The principal difficulty arises from the fact that in some insects the lining of the midgut arises from mesenteron rudiments carried in on the tips of the stomodæal and proctodæal invaginations, whereas in other insects this lining is produced by proliferation from the tips of *unilaminar* stomodæal and proctodæal invaginations. Eastman (1927) and Richards (1932) have suggested that this is only a difference in the time of determination of the functional endoderm. Using maps of prospective significance Richards illus-

trates the difficulty encountered if we consider the functional endoderm as determined before its growth into the definitive midgut in forms in which it arises by proliferation from the unilaminar tips of the stomodæal and proctodæal investigations. He suggests that in such forms it is not determined before this time and that its determination must be a function of the position of the cells concerned.

Reith (1925) reports that the midgut anlagen are practically the only part of the House Fly egg capable of development beyond their prospective significance. In this species more than half of the midgut is formed from one end when either the stomodæal or proctodæal invagination is absent.

2. **Germ Cells:** In certain insects (Honey Bee, moths, etc.) the gonads and presumably also the germ cells originate in the genital ridge of the splanchnic mesoderm. This type has not been studied experimentally during embryonic stages. In certain other insects the germ cells are segregated at the posterior pole of the egg during cleavage (called 'pole cells'). Their further development is more or less independent of the rest of the embryo.

Hegner (1908, 1911) showed that the elimination of the posterior pole from the eggs of Chrysomelid beetles either by pricking and allowing part of the egg contents to flow out or by killing with a hot needle results in an embryo lacking germ cells and possessing certain structural defects. Reith (1925) obtained similar results with the House Fly. Geigy (1931a) reports that killing the posterior pole of *Drosophila* eggs by ultra-violet irradiation during cleavage results in adults whose gonads are composed of only mesodermal elements (*i.e.,* contain no germ cells). Shorter irradiation frequently resulted in unilateral castration. The single gonad might be small, of normal size or larger than normal. To explain these large single gonads he accepts Ruppert's suggestion (1924) that in addition to killing some of the cells the ultra-violet rays cause an adhesiveness of the germ cells so that they stick together during migration into the embryo instead of separating into two gonadal groups. More exacting data on *Drosophila* are given by Howland & Robertson (1934). They dechorionated eggs and killed part or all of the 'pole cells' by carefully localized point cauterization. The sole effect was

partial or total sterility. Therefore the 'pole cells' are not only destined to form the definitive germ cells but they are incapable of being regenerated by an otherwise normal embryo.

3. **Imaginal Discs:** This topic will be treated more fully in Part II. By analysis of *Drosophila* gynandromorphs Sturtevant (1929) shows that the cortical layer is not only determined for the parts of the embryo but also (perhaps secondarily) mapped out for the adult *via* the presumptive imaginal discs. Geigy's results (1931b) made it seem that Sturtevant's data were valid only for prospective significances since Geigy obtained imaginal defects only when he irradiated eggs with ultra-violet light after the differentiation of the larval organs had begun. Geigy therefore advanced the idea of two separate determination periods in Diptera, the first for the embryo, the second for the adult. But Smith (1935) reports similar non-hereditary defects from x-rayed female gametes, and thereby leads to our questioning the validity of Geigy's two periods. Perhaps the discrepancy can be traced to the different types of irradiation used but it seems best to leave it an open question.

4. **Duplication of single organs:** These are produced by the same agents that cause duplications of whole parts. They illustrate two points of interest: (1) that any internal organ, including the nervous system, or any external part is capable of duplication separately or in combination with other parts: and (2) that duplications represent a positive new or additional formation in the sense that the sum of the two duplicated parts exceeds the size of a single normal organ. In fact, the size is increased even when the parts 'heal' so that no duplications occur. To date duplications have been produced only in indeterminate types of eggs and the phenomenon is one of the criteria used to distinguish this type of development (Seidel, Krause).

Cappe de Baillon (1927) and von Lengerken (1928) suggest that some duplications may result from the fusion of two oöcytes whose cortical layers are partially determined at the time of fusion. Positive evidence of this is available only in the phasmids (indeterminate type of egg).

5. **Order of embryonic determination:** In addition to the determination process passing anteriorly and posteriorly from

the thoracic differentiation center there is sometimes a later, secondary determination for specific organ characteristics. The data are from intermediates.

To explain intersexes of the Gypsy Moth Goldschmidt (1927, 1931) postulates that the individuals begin development as one sex, that a physiological change constituting a turning point occurs, and that subsequent development is characteristic of the other sex. All structures finally determined before the time of this change will be of the former sex, all determined later will be of the opposite sex. An intersex is then a 'time mosaic' of male and female parts due to differences in the time of determination of specific organ-types (Shull 1930b). In application it is assumed that in the induced change from one sex to the other the order of determination is the reverse of the order of modification in specimens successively more like the opposite sex. Applying this Goldschmidt finds that the sex of the gonads and abdomen are determined before that of the wings and antennæ. One of the most interesting points is that the onset of histological differentiation of the sexual characters does not necessarily signify that the sex of those organs is finally determined. This is clearly shown by the gonads. These may develop to the point of containing almost mature eggs or sperm and then following sex-reversal have the differentiated germ cells degenerate and be replaced by the differentiation of germ cells of the opposite sex. All this occurs because of the genetic constitution of the cells themselves and presumably is not influenced by hormones.

Shull (1930b, 1931) reports that when the offspring of winged females of the aphid *Macrosiphum* are gradually changed from gamic to parthenogenetic type, the differential features of successive offspring change at different times and rates. The first to change are the color of the antennæ and the color and size of the tibial sensoria, then the body color and reproductive system. Within the reproductive system the collateral glands and seminal receptacles change sooner than the ovarioles. However, when the mother is induced to revert to the production of gamic instead of parthenogenetic offspring, this series of changes instead of occurring in the same order occurs in the reverse order contrary to expectations based upon the time of determination hypothesis. This phenomenon remains unexplained.

XI. SUMMARY

1. The earlier developmental processes in the insect egg have been experimentally studied in species of Orthoptera, Odonata, Coleoptera, Hymenoptera and Diptera. (Section II.)

2. The fundamental processes underlying the determination of polarity and symmetry are unknown. In bilaterally symmetrical eggs, at least, where the main axes are usually coincident with those of the mother, polarity and symmetry must be impressed upon the developing oöcyte, perhaps by extra-oval factors. Secondary influence by the sperm, if any, would be possible only in radially symmetrical eggs. Gravity is not a factor. (Section III.)

3. The phenomena of fertilization are likewise poorly understood. At fertilization eggs are usually in the first meiotic metaphase. In some species the sperm does, in others does not have an activating function. Polyspermy is common. Bi- and trinucleate eggs may develop with correlated effects on the morphology and genetic constitution of the products. Parthenogenesis is widespread and has been induced in some gamic species. (Section IV.)

4. The cleavage nuclei are not restricted as to their destination in the embryo. They divide synchronously for a specific number of divisions but sooner or later heterochronism sets in. They are not the decisive factors in determination and are indeterminate or totipotent at least until the blastoderm stage (except in certain species in which the nuclei entering the germ-tract determinant region are differentiated sooner, and also the vitellophags of certain species). (Section V–A.)

Migration of cleavage nuclei is generally regarded as not autonomous but the result of extrinsic factors in the surrounding egg plasma (flowing or contraction) which are also instrumental in the formation of the inner cortical layer. The origin of these movements is at present a matter of conjecture. (Section V–B.)

Nuclear migration is distinct from blastoderm-cell formation. There is evidence that the latter may be a function of the activation center, predetermined cytoplasm, oöplasmic streaming or nuclear stimulation, these factors varying in importance in different insects. (Section V–C.)

5. Insect eggs may be arranged in a series ranging from indeterminate to determinate types. This series is valid in regard to both the relative time of visible differentiation of the organ anlagen (the sooner visible the more determinate the egg) and the degree of potency or regulative power of the egg parts (greater regulation and later determination in indeterminate eggs). Hence at the time of deposition the egg may be of either the regulative or mosaic type, with subsequent determination in the former occurring either rapidly or gradually and sometimes accompanied by visible differentiation of the unsegmented cytoplasm (the ant). Cell formation probably plays only an indirect role in differentiation. (Section VI.)

6. Two physiological centers may be present in the insect egg: an activation center at the posterior pole and a differentiation center in the presumptive thoracic region. The former has been demonstrated in a damsel fly, two beetles and two ants; the latter is probably present in all insects and has been studied in detail in a damsel fly and bee.

The activation center confers upon the egg the ability to undergo development, but its rôle in embryonic determination is unknown. It is not morphologically distinct, may or may not require interaction with cleavage nuclei, and releases a substance which spreads anteriorly through the egg. This product seemingly stimulates the differentiation center to function. (Section VII–A.)

The differentiation center normally coincides with the region where differentiation of the embryonic rudiment begins in the presumptive thoracic region. Reduction of the size of the egg system results in a displacement of the region of first visible differentiation to a position relative to the new whole, but the physiological center is seemingly retained at its original site. This physiological center is essential for differentiation and, where analyzed, functions as a dynamic center whence proceed waves of contraction in the yolk system which control differentiation and regulation. In the bee it is a "concentration center of potencies." The embryonic differentiation center is also concerned in the development of adult structures in *Drosophila*. (Section VII–B.)

The entire egg must be regarded as a system in which not only the embryonic part and its included factors but also the extra-embryonic parts, especially the yolk system, are instrumental in determination, differentiation and regulation. Determination is carried out by a harmonious alternating series of interacting dynamic processes (flowing and contraction) and material reactions, the former involving the egg system as a whole and enabling the reactions of more or less definite centers. Hence developmental processes are not primarily the result of the functioning of 'centers' but rather of the relations existing in the egg as a whole. (Section VII–C.)

On this basis a comparison can be drawn between insect and amphibian eggs, but at present the superficial resemblances scarcely allow direct analogy of the vertebrate organizer with either the insect activation center or differentiation center or both. (Section VII–D.)

7. Blastokinesis, at least in the grasshopper, is accomplished by vigorous movements of the embryo itself. (Section VIII.)

8. Maps have been made of the general embryonic anlagen-plan of the damsel fly (by defect experiments which show potencies rather than true promorphology) and of the potencies of the various levels of the Honey Bee egg (by constriction experiments). In the determinate eggs of Diptera presumptive anlagen arise at the points where the corresponding organs first become visible. (Section IX.)

9. There are only incidental experimental data on organ-formation, the mosaic stage of differentiation having still to be analyzed in detail. Notes are given on the endoderm, germ cells, imaginal discs, organ duplications and order of embryonic determination. In certain insects in which some of the cleavage nuclei become segregated at an early stage at the posterior pole of the egg ('pole cells') it has been conclusively proved that these cells give rise to the definitive germ cells. (Section X.)

XII. LITERATURE

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